

We Claim:

- 1) A DNA construct having a formula
pY – SP – B(1-29)-A(1-21),
where A) pY is any promoter in yeast, B) SP encodes a signal peptide region that enables
5 the secretion of polypeptides expressed in yeasts, and is derived from either
Schwanniomyces occidentalis glucoamylase signal peptide sequence or from *Carcinus*
maenas crustacean hyperglycemic hormone signal peptide sequence, and lies to the N-
terminus of the insulin peptide region B(1-29)-A(1-21) and C) B(1-29)-A(1-21) encodes,
upon expression, the insulin peptide region in which B(1-29) is the B chain of insulin
10 from amino acid 1 to amino acid 29, A(1-21) is the A chain of insulin from amino acid 1
to amino acid 21, and that the amino acid 29 of the B chain directly connects, by means
of a peptide bond, the amino acid 1 of the A chain and the expression of SP – B(1-29)-
A(1-21) region is under the control of the promoter - pY.
- 2) A DNA construct according to claim 1 where the SP is derived from *Schwanniomyces*
15 *occidentalis* glucoamylase signal peptide sequence.
- 3) A DNA construct according to claim 1 where the SP is derived from *Carcinus maenas*
crustacean hyperglycemic hormone signal peptide sequence.
- 4) A DNA construct according to claim 2 in which the SP carries a kex protease cleavage
site.
- 20 5) A DNA construct according to claim 3 in which the SP carries a kex protease cleavage
site.
- 6) A DNA construct according to claim 2 in which the SP does not carry any kex
protease cleavage site.
- 7) A DNA construct according to claim 3 in which the SP does not carry any kex
25 protease cleavage site.
- 8) A DNA construct according to claim 6 in which the SP has a single methionine residue
placed such that it is just adjacent and N-terminus to the polypeptide encoded by the
insulin peptide region B(1-29)-A(1-21).
- 9) A DNA construct according to claim 7 in which the SP has a single methionine residue
30 placed such that it is just adjacent and N-terminus to the polypeptide encoded by the
insulin peptide region B(1-29)-A(1-21).

- 10) A DNA construct according to claim 6 in which the SP has either a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).
- 11) A DNA construct according to claim 7 in which the SP has either a single Arginine or
5 a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).
- 12) A polypeptide SP-B(1-29)-A(1-21) B(1-29)-A(1-21), where SP is a signal peptide region that enables the secretion of polypeptides expressed in yeasts and is derived from either *Schwanniomyces occidentalis* glucoamylase signal peptide sequence' or from
10 *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequence, and lies to the N-terminus of the insulin peptide region B(1-29)-A(1-21), and further where B(1-29) is the B chain of insulin from amino acid 1 to amino acid 29, A(1-21) is the A chain of insulin from amino acid 1 to amino acid 21, and the amino acid 29 of the B chain directly connects, by means of a peptide bond, the amino acid 1 of the A chain.
- 15 13) A polypeptide according to claim 12 where the SP is derived from *Schwanniomyces occidentalis* glucoamylase signal peptide sequence.
- 14) A polypeptide according to claim 12 where the SP is derived from *Carcinus maenas* crustacean hyperglycemic hormone signal peptide sequence.
- 15) A polypeptide according to claim 13 in which the SP carries a kex protease cleavage
20 site.
- 16) A polypeptide according to claim 14 in which the SP carries a kex protease cleavage site.
- 17) A polypeptide according to claim 13 in which the SP does not carry any kex protease cleavage site.
- 25 18) A polypeptide according to claim 14 in which the SP does not carry any kex protease cleavage site.
- 19) A polypeptide according to claim 17 in which the SP has a single methionine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

- 20) A polypeptide according to claim 18 in which the SP has a single methionine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).
- 21) A polypeptide according to claim 17 in which the SP has either a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).
- 22) A polypeptide according to claim 18 in which the SP has either a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).
- 23) A DNA construct according to claim 1 in which the promoter, pY, is of yeast origin.
- 24) A DNA construct according to claim 23 in which the promoter, pY, is either the methanol oxidase promoter (MOX-P) or Formaldehyde dehydrogenase promoter (FMDH-P) or Formate dehydrogenase promoter (FMD-P) or Dihydroxyacetone synthase promoter (DHAS-P).
- 25) A process for the expression of insulin in yeasts which consists of transforming the said yeast with a plasmid that carries the DNA construct of claim 1, culturing the said transformed yeasts in an appropriate culture and isolating the insulin containing polypeptide from the culture medium.
- 26) A process according to claim 25 where the yeast is selected from genera *Hansenula*, *Saccharomyces*, *Pichia*, *Kluyveromyces*.
- 27) A process according to claim 26 where the yeast is *Hansenula polymorpha*.
- 28) A DNA construct of claim 1 in which B(1-29) is the B chain of human insulin from amino acid 1 to amino acid 29, A(1-21) is the A chain of human insulin from amino acid 1 to amino acid 21.
- 29) Process for the isolation, purification and conversion to native insulin, of the polypeptides of claims 15 consisting of the following steps:
- Clarification of the culture supernatants containing the above polypeptides.
 - Subjecting the clarified culture supernatants to cation exchange chromatography.
 - Isoelectric precipitation of the cation exchange chromatography derived polypeptides.
 - Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.

- e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.
- f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.
- g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was
5 purified on a reverse phase HPLC column.
- h) Isoelectric precipitation of the purified insulin.
- 30) A process according to claim 29 where any two steps are performed in sequence.
- 31) Process for the isolation, purification and conversion to native insulin, of the polypeptides of claim 16 consisting of the following steps:
 - 10 a) Clarification of the culture supernatants containing the above polypeptides.
 - b) Subjecting the clarified culture supernatants to cation exchange chromatography.
 - c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.
 - d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.
 - 15 e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.
 - f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.
 - g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.
 - 20 h) Isoelectric precipitation of the purified insulin.
 - 32) A process according to claim 31 where any two steps are performed in sequence.
 - 33) Process for the isolation, purification and conversion to native insulin, of the polypeptides of claim 21 consisting of the following steps:
 - a) Clarification of the culture supernatants containing the above polypeptides.
 - 25 b) Subjecting the clarified culture supernatants to cation exchange chromatography.
 - c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.
 - d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.
 - e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase
30 chromatography.
 - f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.

- g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.
 - h) Isoelectric precipitation of the purified insulin.
- 34) A process according to claim 33 where any two steps are performed in sequence.
- 5 35) Process for the isolation, purification and conversion to native insulin, of the polypeptides of claim 22 consisting of the following steps:
- a) Clarification of the culture supernatants containing the above secreted polypeptides.
 - b) Subjecting the clarified culture supernatants to cation exchange chromatography.
 - c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.
 - 10 d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.
 - e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.
 - f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.
 - 15 g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.
 - h) Isoelectric precipitation of the purified insulin.
- 36) A process according to claim 35 where any two steps are performed in sequence.